DATA SHEET

TURBIDIMETRIC IMMUNOASSAY FOR ULTRASENSITIVE
DETERMINATION OF C-REACTIVE PROTEIN
SUMMARY

C-reactive protein (CRP), the classical acute phase protein is an extremely valuable marker for underlying systemic inflammation. The median value for serum CRP in apparently healthy adults is ~ 0.08 mg/dl, the 90th centile of distribution in such subjects is ~ 0.03 mg/dl. The baseline values for CRP in a healthy individual remain stable over a long period's time. The baseline serum concentration of CRP predicts the risk of future myocardial infarction and stroke independent of other risk factors, in apparently healthy subjects. Increased values of CRP below 0.5 mg/dl previously considered to be within the reference interval are strongly associated with increased risk of atherothrombotic events. Several prospective studies suggest that in apparently healthy individuals, as the concentration of CRP increases from greater than 0.055 to 0.211 mg/dl, the probability for developing AMI increases significantly from a factor of 1 to 2.9. Apparently healthy individuals in the highest quartile (the upper 25%) of the above mentioned range have 2-3 times higher risk of developing subsequent atherosclerotic diseases compared to those in the lowest quartile. Simultaneous measurements of ultrasensitive CRP and total HDL cholesterol predict future vascular risk better than lipid measurements alone.
Such low levels of CRP in apparently healthy adults can be determined by ultrasensitive immunoassays such as ®Quantia-CRP US .

PRESENTATION

REF	10730050	10730150
R1	2 x 10 ml	2 x 30 ml
R2	5 ml	15 ml
CAL	1 ml	1 ml

REAGENT
®

1. Quantia-CRP US Activation Buffer(R1): Ready to use ®
2. Quantia-CRP US Latex Reagent (R2): Ready to use uniform suspension of polystyrene latex particles coated with Anti-CRP antibodies. ®
3. Quantia-CRP US Calibrator,: A lyophilized preparation of serum equivalent to the statedamount of CRP on a mg/dl ®basis, when hydrated appropriately. The Quantia-CRP US calibrator is traceable to the W.H.O. International Reference Standard (85/506) for Human C-reactive protein Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

REAGENT STORAGE AND STABILITY
1. Store the reagents at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagent, activation buffer and calibrator is as per the expiry datementioned on the respective vial label.
3. The reconstituted Quantia-CRP US calibrator is stable for 7 days at 2- 8°C and 48 hours at 25°C-30°C (RT.).

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4. The working reagent for Quantia-CRP US can be prepared by mixing R2 and R1 in the ratio 1:5.
5. The mixed stability of the working reagent (R1 + R2) is 7 days when stored at 2-8°C.
PRINCIPLE®Quantia-CRP US is a turbidimetric immunoassay for the ultrasensitive determination of C-reactive protein in human ®serum and is based on the principle of agglutination reaction. The test specimen is mixed with Quantia-CRP US latex reagent and activation buffer and allowed to react. Presence of CRP in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at wavelength between 505 578 nm. The increase in turbidity corresponds to the concentration of CRP in the test specimen.

NOTE
1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagents that are derived from human source have been tested for HBsAg and Anti-HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagents contain 0.1% Sodium Azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water
4. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagents be verified using known controls periodically.
5. Gently mix the Quantia-CRP US latex reagent well before use to disperse the latex particles uniformly to improve test performance.
6. The working reagent should mixed gently.
7. Do not use vortex mixers for mixing reagents. Gently mix the reagents and samples during test procedures.
8. The Quantia-CRP US assay is recommended only for analyzers with cuvette mode. Though any semi automated analyzer with appropriate programming facility can be used, for best results it is recommended to use Quantiamate analyzer. Fully automated analyzers may be used, provided the reagent has been standardized on the system.
9. Calibrators of different manufacturers must not be used with Quantia-CRP US reagents.
10. The calibration curve must be validated periodically with known controls such as SEROQUANT CRP US (Ref: 11107001 & 11108001).
11. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
12. The procedures mentioned in this pack insert are based on a minimum reading volume of 500m l (0.5 ml). In case of instruments where minimum volume required for reading absorbance is 1.0 ml, use double the quantity of reagents and samples mentioned in the test procedure.

SPECIMEN COLLECTION AND PREPARATION
No special preparation of the patient is required prior to specimen collection by approved techniques. Only serum should be used for testing. Should a delay in testing occur, store the samples at 2 - 8°C. Samples can be stored for upto a week at 2 - 8°C, provided they are not contaminated. Do not use hemolysed, icteric, or highly turbid serum. Turbid or particulate serum samples must be clarified by centrifugation at 2000 rpm for 15 minutes. Use the clear

supernatant for testing.

ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 505 to 578 nm wavelength filters and cuvette mode, stopwatch, well calibrated micropipettes, disposable tips, isotonic saline, particulate free distilled water, test-tube rack, incubator/ waterbath set at 37°C optically clean disposable/glass semi micro cuvettes.

Note: Though any filter between the wavelengths 505-578 can be used, optimum results are obtained with a filter with 546 nm wavelength.

TEST PROCEDURE

Bring reagents and samples to room temperature before use.

Assay conditions:

Wavelength	546 nm
Reaction Temperature	37°C
Cuvette	1 cm path length

Method for preparation of CRP US® Calibration Curve

The **Quantia-CRP US®** calibrator must be reconstituted exactly with 1 ml of distilled water, wait for 5 minutes, gently swirl the vial till the solution attains homogeneity. Once reconstituted it is ready to use for preparation of the CRP calibration curve.

The Concentration of CRP (S) in the reconstituted calibrator is as mentioned at the end of this package insert.

Dilute the reconstituted calibrator serially as mentioned below for preparation of calibration curve.

Test Tube No.	1	2	3	4	5	6	7
Calibrator dilution No.	D1	D2	D3	D4	D5	D6	D7
Vol. of Saline in µl	-	100	100	100	100	100	100
Vol. of Calibrator (S) in µl	100	100	100	100	100	100	100
Conc. of CRP in mg/dl	1.0	0.50	0.25	0.125	0.062	0.031	0.015

Five dilutions of the calibrator including the highest concentration1.0 mg/dl (D1) and the lowest concentration 0.015 mg/dl (D7) of the measuring range must be used for the preparation of the calibration curve. Select any of the three dilutions from D2 to D6 in addition to D1 and D7 to prepare the calibration curve.

Test procedure for preparation of calibration curve

(Note: During calibration on instrument with programming facilities, increasing concentration of the standards must be used for preparing calibration curve.)

1. Zero the instrument with distilled water
2. Pipette and 400 ml of Quantia-CRP US activation buffer (R1) and 100m l of Quantia-CRP US Latex reagent (R2) in the measuring cuvette. Mix well and incubate for 5 minutes at 37°C. OR Pipette 500 ml of the Quantia-CRP US working reagent in the measuring cuvette. Mix well and incubate for 5 minutes at 37°C.
3. Add 5m l of calibrator D1, mix gently and start the stopwatch simultaneously.
4. Read absorbance (A1), exactly at 10 seconds, and absorbance (A2) again at the end of exactly four minutes.
5. Repeat steps No. 2-4 for each of the diluted calibrator selected for preparing the calibration curve.
6. Calculate DA (A2-A1) for each of the diluted calibrator selected for preparing the calibration curve. Plot a graph of DA

versus concentration of CRP on the graph paper provided with the kit.

“The calibration curve” so obtained is valid only for the same lot of Quantia-CRP US reagent.

Test procedure for specimen For determination of CRP concentration in the test specimen;

1. Follow steps 2- 4 as mentioned in the above procedure for calibration curve using the test specimen in place of the calibrator.
2. Calculate DA (A2-A1) for the test specimen.

VALIDATION CRITERIA
If the DA of the test specimen is less than DA obtained for the calibrator of highest concentration (D1) then the concentration of CRP in the test specimen can be determined directly by interpolating DA of the test specimen from the calibration curve.
If the DA of the test specimen is higher than DA of the calibrator with highest concentration (D1) then the test has to be rerun by carrying out serial dilutions (such as 1:2, 1:4 etc) of test specimen till the DA of the diluted test specimen is less than DA of D1.
The CRP concentration for such samples can be determined as mentioned below in calculations.

CALCULATIONS
1. Interpolate DA of the diluted test specimen on the calibration curve and obtain the CRP concentration 'C' of the diluted test specimen.
2. Multiply the CRP concentration 'C' with the dilution factor (F) of the test specimen for obtaining the concentration of CRP in the neat test specimen.
Concentration of CRP in the neat test specimen in mg/dl = C x F (Where 'F' is the dilution factor of the test specimen, for e.g. 2 for 1:2 dilution of test specimen and so on.)

SPECIFIC PERFORMANCE CHARACTERISTICS
Measuring rangeThe Quantia-CRP US reagent has been designed to measure CRP concentrations in the range 0.015-1.0 mg/dl and is linear within the measuring range.
Detection limit / Analytical Sensitivity
Detection limit: 0.015 mg/dl
The detection limit represents the lowest measurable CRP concentrations that can be distinguished from zero.
Prozone limit No prozone effect was observed upto a CRP concentration of 2.0 mg/dl.

Precision

Intra-assay precision	n	Mean mg/dl	SD	CV (%)
Sample 1	15	0.029	0.002	6.02
Sample 2	15	0.349	0.028	8.17
Sample 3	15	0.797	0.058	7.28

Inter-assay precision	n	Mean mg/dl	SD	CV (%)
Sample 1	15	0.029	0.002	5.42
Sample 2	15	0.346	0.017	5.03
Sample 3	15	0.812	0.035	4.27

Interference

No interference was observed with:

Interference factor	No interference upto
Albumin	10 g/dl
Bilirubin	50 mg/dl
Glucose	500 mg/dl
Haemoglobin	500 mg/dl
Triglycerides	1000 mg/dl
RF	120IU/ml

Accuracy

25 samples ranging from 0.015-1 mg/dl CRP were assayed with **Quantia-CRP US®**. The results obtained with **Quantia-CRP US®** (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. The correlation coefficient (r) was 0.99 and the regression equation (Passing and Bablok) was y = 0.072 + 0.9681 x

REFERENCE VALUES

MALES		
CRP in mg/dl	Relative risk factor	
	Future MI	Future Stroke
> 0.211 mg/dl	2.9	1.9
0.115 - 0.210	2.6	1.9
0.056 - 0.114	1.7	1.7
< 0.055	1.0	1.0

FEMALES	
CRP in mg/dl	Relative risk factor
	Future MI or stroke
> 0.73	5.5
0.38 - 0.73	3.5
0.15 - 0.37	2.7
< 0.15	1.0

The above mentioned reference values are for guidance only. It is recommended that each laboratory should define its
own reference range for relevant population.

REMARKS

1. Usage of well-calibrated equipment and accessories and procedures is critical for achieving correct results.
2. When DA obtained for the test specimen is greater than the DA of the standard with highest concentration then, it indicates that the concentration of CRP in the test specimen is beyond the measuring range of the Quantia-CRP US assay. Such specimens should be rerun with further dilutions.
3. When using CRP to assess risk of cardiovascular and peripheral vascular disease, measurements should be compared to previous values. Recent medical events resulting in tissue injury, infections and inflammation, which may cause elevated CRP levels, should also be considered when interpreting results.

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4. Markedly lipemic, hemolysed, and contaminated serum samples could produce non-specific CRP values.
5. Use of plasma rather than serum can lead to erroneous CRP values.
6. Elevated levels of CRP are found to be present after the 1st trimester of pregnancy and persists until delivery.
7. CRP levels are elevated in women who are on oral contraceptives.
8. The commonly used anti-inflammatory drugs or immunosuppressive drugs, including steroids do not affect CRP response, unless the disease activity is affected and it covers an exceptionally broad incremental range upto 3000 times.
9. Do not read results beyond four minutes.
10. Since CRP production is a non-specific response to tissue injury, it is recommended that results of the test should be correlated with clinical findings to arrive at the final diagnosis.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

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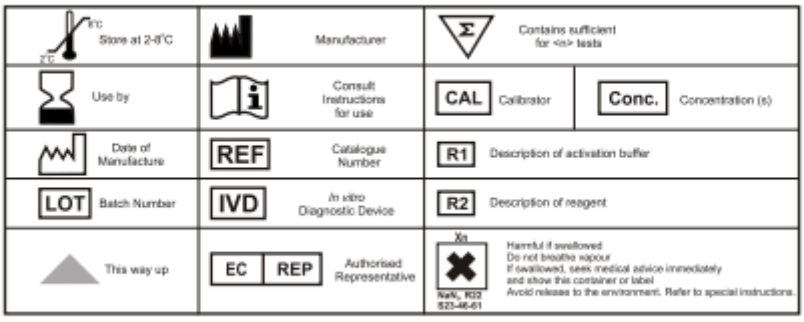

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